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(71) Applicant: LUITPOLD PHARMACEUTICALS, INC. [US/US]; One Luitpold Drive, Shirley, NY 11967 (US).

(72) Inventors: LAWRENCE, Richard, P.; 94 Youngs Avenue, Baiting Hollow, NY 11933 (US). LANGE, Ralf, A.; P.O. Box 1967, Amagansett, NY 11930 (US). HELENEK, Mary Jane; 19 Jean Place, Syosset, NY 11791 (US).

(74) Agent: MORGAN, LEWIS & BOCKIUS LLP; 1800 M Street, NW, Washington, DC 20036 (US).

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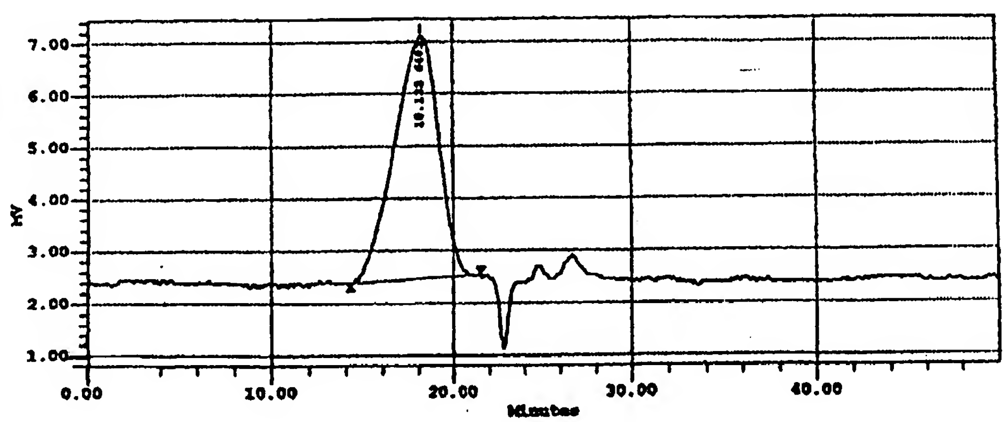

(57) Abstract

Compositions and methods for the treatment of Lyme disease associated joint and arthritic symptoms with polysulfated glycosaminoglycans are described.

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Treatment of Lyme Disease with Polysulfated Glycosaminoglycan Formulations

by

Richard P. Lawrence, Ralf A. Lange and Mary Jane Helenek

FIELD OF THE INVENTION

This invention relates to the treatment of Lyme disease with polysulfated glycosaminoglycan compositions, particularly for the treatment of connective tissue disorders, including arthritis and inflammatory joint conditions associated with Lyme disease.

BACKGROUND

The present invention relates to a polysulfated glycosaminoglycan (PSGAG) composition useful for the treatment of Lyme disease associated arthritis and for prophylactic treatment against Lyme disease associated arthritis and joint disorders in patients. Prior to the present invention, however, the use of PSGAGs in the treatment of Lyme disease had not been suggested.

1. Lyme Disease

Lyme disease was first recognized in the United States in 1975, after an outbreak of arthritis near Lyme, Connecticut. Since then reports of Lyme disease have increased dramatically, from 491 cases in 1982 to 12,801 in 1997, and has quickly become the most common vector-transmitted illness in the USA (National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases: Public Information Guide, ww.cdc.gov/ncidod/diseases/lyme/lyme.htm, March 3, 1999 and Simon *et al., Immunol Today* (1991) 12(1):11-16). In endemic areas, its occurrence is extremely common in children (Prose *et al., Semin Dermatol* (1992) 11(1):31-36) and, in fact, Lyme disease has increasingly become a health concern in Europe.

Lyme disease is spread by the bite of ticks of the genus *Ixodes* that are infected with *Borerrelia burgdorferi*. The disease is characterized as a multisystemic disorder, in which the invasive spirochete has a predilection for collagenous tissue which results in one of the major clinical manifestation of the disease; arthritis (Grab *et al., FEMS Microbiol Lett* (1996) 144(1):39-45). Early symptoms and signs of Lyme disease are best recognized by early skin lesions, *erythema chronicum migrans* (ECM), that are usually accompanied by chills and fever, headache and muscle and joint pain. Arthritis occurs in about half (51%) of patients with ECM within weeks to months of onset (Steere *et al., Ann Intern Med* (1987) 107(5):725-731). Intermittent swelling and pain in a few large joints, especially the knee, typically recur for several years (Merck Manual (Berkow *et al., eds*) 1992, 16th edition, §1, pages 154-157, Merck Research Laboratories, Merck & Co., Inc., Rahway, N.J.).

Lyme disease is usually treated by antibiotics, however, symptoms may continue to recur, including in about 10% of patients who develop chronic (unremittant for 6 months or more) knee involvement (National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases: Public Information Guide, *www.cdc.gov/ncidod/diseases/lyme/lyme.htm*, March 3, 1999 and Merck Manual (Berkow *et al., eds*) 1992, 16th edition, § 1, pages 154-157, Merck Research Laboratories, Merck & Co., Inc., Rahway, N.J.).

Many pathogenic microbes are known to cause infections in joints or bones. These forms of reactive arthritis may involve adherence to mammalian host tissues through sulfated carbohydrates, a pathway which has been suggested to be an important trait in microbial pathogenesis. Such pathogens include mycobacteria (Menozzi *et al., J Exp Med* (1996) 184(3):993-101) candida (Dupont *et al., J Infect Dis* (1985) 152(3):577-591), gonococci (Chen *et al., J Exp Med* (1995) 182(2):511-517), staphylococci (Gallenga *et al., Opthalmologica* (1998) 212(3):184-187) and Haemophilus influenzae (Noel *et al., Infect Immun* (1994) 62(9):4028-4033). However, the epidemiology of septic arthritis suggests that its occurrence is more often associated with

disseminated infections (Dupont *et al., J Infect Dis* (1985) 152(3):577-591), immune compromised hosts (Merck Manual (Berkow *et al., eds*) 1992, 16th edition, § 10, pages 1342-1343, Merck Research Laboratories, Merck & Co., Inc., Rahway, N.J.), secondary infection associated with rheumatoid arthritis (*Ibid.*), secondary effects due to trauma or surgery *(Ibid.*) and poor overall health due to illicit drug use (Rich *et al., Arthritis Rheum* (1996) 39(7):1172-1177 and Dupont *et al., J Infect Dis* (1985) 152(3):577-591). For example, while disseminated gonococcal infections (DGI) account for up to two-thirds of septic arthritis seen in the United States, it only effects about 1% of persons with gonorrhea (in Cecil, Textbook of Medicine (Bennett *et al., eds*), 1996, 20th edition, Chapter 239, page 1473 and Chapter 315, page 1701,W.B. Saunders Company, Philadelphia, PA). In other words, the prevalence in the population for developing infective arthritis is too low to warrant prophylactic anti-arthritis paradigms as a treatment modality.

B. burgdorferi adhere to mammalian cells *in vitro*, and studies indicate that one mode of attachment is mediated by a glycosaminoglycan receptor attaching to surface-exposed proteoglycans on mammalian cells (Isaacs RD., *J Clin Invest* (1994) 93(2):809-19). Furthermore, studies have suggested that *B. burgdorferi* possess a collagenolytic activity which might be involved in the pathogenesis of Lyme arthritis (Grab *et al., FEMS Microbiol Lett* (1996) 144(1):39-45). Along with ECM, which occurs in 85-95% of seropositive patients (Merck Manual (Berkow *et al., eds*) 1992, 16th edition, § 1, pages 154-157, Merck Research Laboratories, Merck & Co., Inc., Rahway, N.J.; Ciesielski *et al., Rev Infect Dis* (1989) 11 Suppl 6:S1435-S1441; Steere *et al., Ann Intern Med* (1987) 107(5):725-731; and Lane *et al., Ann NY Acad Sci* (1988) 539:192-203), arthritis is a key epidemiological event for Lyme disease (Merck Manual (Berkow *et al., eds*) 1992, 16th edition, § 1, pages 154-157, Merck Research Laboratories, Merck & Co., Inc., Rahway, N.J., National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases: Public Information Guide, ww.cdc.gov/ncidod/diseases/lyme/lyme.htm, March 3, 1999, and Du Chateau *et*

al., Infect Immun (1996) 64(7):2540-2547). In fact the occurrence of arthritis as a symptom of the disease ranges from 50-62% (Merck Manual (Berkow *et al., eds*) 1992, 16th edition, § 1, pages 154-157, Merck Research Laboratories, Merck & Co., Inc., Rahway, N.J. and Huppertz *et al., Arthritis Rheum* (1995) 38(3):361-368). Therefore, in contrast to disseminated infections associated with pathogens of septic arthritis, the prevalence in the population for developing Lyme disease associated arthritis is high, thus, prophylactic anti-arthritis paradigms are warranted and desired.

2. Immunopathogenesis and Treatment of Lyme Disease

Both infectious and immunological mechanisms are important factors in the pathogenesis of Lyme disease (Goodwin *et al., Clin Pharm* (1990) 9(3):192-205). Animal, human and *in vitro* studies have demonstrated that *B. burgdorferi* is capable of inducing a strong immune response, suggesting that autoimmune mechanisms may play a significant role in treatment-resistant chronic Lyme disease (Hu *et al., I Clin Immunol* (1997) 17(5):354-365). It seems that during the onset of Lyme arthritis, the balance between TH1 and TH2 is modulated in the choice between inflammatory and antibody mediated immune responses (Muller *et al., Springer Semin Immunopathol* (1998) 20(1-2):181-196). These include the induction of chemokines, adhesion molecules and transcription factors which produce an environment for leukocyte recruitment which may explain the autoimmune mechanisms that coincide with spirochetal persistence and exacerbation of injuries to joints associated with Lyme arthritis (Anguita *et al., Infect Immunol* (1997) 65(10):4334-4336; Garcia-Monco *et al., Rheum Dis Clin North Am* (1989) 15(4):711-26; and Sigal LH., *Ann Rev Immunol* (1997) 15:63-92).

It is clear that T cells and macrophages have a direct, effector role in the pathogenesis of Lyme arthritis (Du Chateau *et al., Infect Immun* (1996) 64(7):2540-2547 and Sigal LH., *Ann Rev Immunol* (1997) 15:63-92). In animal studies it has been shown that severe destructive arthritis was induced in

recipients of macrophages from *B. burgdorferi* vaccinated animals (Du Chateau *et al., Infect Immun* (1996) 64(7):2540-2547). Thus, inhibition of specific cytokines and lymphokines (*e.g.*, IL-12 inhibitors, CD40 ligand inhibitors, IL-1 inhibitors, Nf-kappa B inhibitors), may in fact be useful in combination with therapeutic compositions for treatment-resistant chronic Lyme disease associated arthritis.

Current treatment for Lyme disease involves the use of antibiotics, such as oral doxycyclin, oral amoxicillin and probenecid. For relief of arthritis-related symptoms, aspirin or other non-steroidal anti-inflammatory drugs (*e.g.*, idomethacin) may be used.

3. Rheumatoid Arthritis and Current Treatments

Current treatments of arthritis involve the combined use of rest, nutrition and drugs. Conservative treatments within the first year of disease include nonsteroidal anti-inflammatory drugs (NSAID), such as salicylates (*e.g.*, aspirin). These may also include, indomethacin, ibruprofin, naproxen and others. If the disease progresses and NSAIDs are ineffective or not well tolerated, gold compounds (in addition to NSAIDs) may be administered. Penicillimine also can also be given at this stage if gold treatments are unsatisfactory.

Moderate to severe arthritis requires, as a first treatment, the use of compounds such as hydroxychloroquine and sulfasalazine. When severe symptoms occur, corticosteroids are the most beneficial, however, such benefits diminish with time and such compounds do not prevent the progression of joint destruction. During severe active arthritis, cytotoxic or immunosuppressive drugs such as methotrexate, cyclophosphamide and azathioprine are used. These compounds have many side effects and must be used with intensive monitoring.

4. Polysulfated Glycosaminoglycans

Polysulfated glycosaminoglycan (PSGAG) is a semi-synthetic glycosaminoglycan (GAG) prepared by extracting GAGs from cartilage, for example, bovine tracheal cartilage, and then polysulfating the GAGs. GAGs are polysaccharides composed of repeating disaccharide units. The GAG present in PSGAG is principally composed of chondroitin sulfate, containing about 3 to 4 sulfate esters per disaccharide unit. At therapeutic doses, PSGAG has several biochemical effects on injured or degenerative joints and has been classified as a chondroprotective compound, because PSGAG has been found to be incorporated in the cartilage matrix upon injection.

PSGAG has also been shown to inhibit various catabolic enzymes which degrade glycosaminoglycans competitively, including elastase, stromelysin, metalloproteases, cathepsin Bi, hyaluronidases and collagenases. PSGAG has also been demonstrated to indue synthesis of collagen, proteoglycans and hyaluronic acid by chondrocytes and synoviocytes. Further, anti-inflammatory activity has been demonstrated for these molecules. An *in vitro* study of proteoglycan binding of *B. burgdorferi* found that glycosaminoglycans inhibited the attachment of the spirochete to certain mammalian cells (Leong *et al., Infect Immunol* (1998) 66(3):994-999). However, these same molecules do not share the inhibitory properties of PSGAG (see, for example, Kobiashi *et al., Biochim Biophys Acta* (1998) 1425(2):369-376).

5. Polysulfated Glycosaminoglycans and Uses Thereof to Treat Arthroses

The use of GAGs in the treatment of non-Lyme disease related connective tissue disorders also has been disclosed in the medical and patent literature, including methods of treating damage corneal, uterine or cartilage tissues and/or treating rheumatism (see Klundas, U.S. Patent No. 5,036,056 and Akoi *et al.,* U.S. Patent No. 5,470,578). However, these disclosure are limited to repair and/or replacement of GAG loss in the extracellular matrix, and the administration of GAGs would not have been expected to inhibit, for example,

the contributory effects of elastases and chondroitinases on connective tissue degeneration as these GAGs themselves would be substrate for such enzymes.

The literature also describes both the preparation and use of sulfated chondroitin sulfate, including the use of such polysulfated chondroitin for therapy in humans and animals as an antiarthritic treatment (see Wolf U.S. Patent No. 4,534,066). There have been a number of clinical trials of PSGAG in the treatment of osteoarthritis in humans reported in the literature.

PSGAG previously was marketed in Europe for the treatment of degenerative joint disorders, and reportedly was most effective as long as there is no extensive full thickness loss of articular cartilage. The intramuscular treatment regimen in humans was 50 mg twice weekly for up to 15 injections. That European formulation of PSGAG was taken off the market in 1994 because two deaths occurred in the treated patient group. However, it is believed by the present inventors that PSGAGs may be safely administered to humans with appropriate care. For example, it is now known that PSGAG should not be administered intra-articularly in the presence of active joint inflammation.

Two PSGAG veterinary products are marketed by the assignee of the present application. The ADEQUAN® Canine formulation is recommended for intramuscular injection for the control of signs associated with non-infectious degenerative and/or traumatic arthritis of canine synovial joints. Similarly, the ADEQUAN® i.m. formulation is recommended for the intramuscular treatment of non-infectious degenerative and/or traumatic joint dysfunction and associated lameness of the carpal and hock joints in horses.

The ADEQUAN® CANINE formulation, is a formulation of PSGAG for intramuscular administration to dogs. It is sold in a solution of 100 mg/mL in a 5 mL preserved solution. Each mL of ADEQUAN® Canine contains 100 mg of PSGAG, 0.9% v/v benzyl alcohol as a preservative, and water for injection q.s. to 1 mL. Sodium hydroxide and/or hydrochloric acid are added when necessary to adjust pH. A second product, ADEQUAN® i.m.

formulation, is a formulation of PSGAG for intramuscular use in horses, which is sold in a solution of 500 mg/5 mL. Each 5 milliliters of the ADEQUAN® i.m. formulation contains 500 mg of PSGAG and water for injection q.s. Sodium hydroxide and/or hydrochloric acid may be added when necessary to adjust the product's pH. Sodium chloride may be added to adjust tonicity.

As described in the package inserts (NADA #141-038, Iss. 4/97 and NADA #136-383, Rev. 04/96) for these products, PSGAG is chemically similar to the glycosaminoglycans in articular cartilage matrix. PSGAG is a potent proteolytic enzyme inhibitor and diminishes or reverses the pathologic processes of traumatic or degenerative joint disease which result in a net loss of cartilage matrix components. PSGAG improves joint function by reducing synovial fluid protein levels and increasing synovial fluid hyaluronic acid concentration, for example, in traumatized equine carpal and hock joints.

The specific mechanism of action of the ADEQUAN® formulations in canine joints or equine joints is not known. PSGAG is characterized as a "disease modifying osteoarthritis drug". Experiments conducted *in vitro* have shown that PSGAG inhibits certain catabolic enzymes that have an increased activity in inflamed joints, and enhances the activity of some anabolic enzymes. For example, PSGAG has been shown to significantly inhibit serine proteinases, which have been demonstrated to play a role in the Interleukin-1 mediated degradation of cartilage proteoglycans and collagen. PSGAG is reported to be an inhibitor of Prostaglandin E2 (PGE2) synthesis. PGE2 has been shown to increase the loss of proteoglycan from cartilage. PSGAG also has been reported to inhibit some catabolic enzymes such as elastase, stromelysin, metalloproteases, cathepsin B1, and hyaluronidases, which degrade collagen, proteoglycans, and hyaluronic acid in degenerative joint disease.

The anabolic effects of PSGAG that have been reported include the compound's ability to stimulate the synthesis of protein, collagen, proteoglycans, and hyaluronic acid in various cells and tissues *in vitro*. Cultured human and rabbit chondrocytes have shown increased synthesis of

proteoglycan and hyaluronic acid in the presence of PSGAG. PSGAGs have shown a specific potentiating effect on hyaluronic acid synthesis by synovial membrane cells *in vitro*.

In contrast to Lyme disease related joint involvement, other arthritic and connective disease disorders (*e.g.*, rheumatoid arthritis) do not have known specific etiologies. Thus, *e.g.*, physicians often do not recognize which parts of the population will spontaneously develop rheumatoid or osteoarthritis until the onset of symptoms (*i.e.*, joint pain and swelling). Hence, there would not appear to be a prophylactic indication, for GAGs for osteoarthritis or rheumatoid arthritis. On the other hand, the prevalence of the population that will develop Lyme disease associated joint involvement, such as arthritis, is highly correlated with both *B. burgdorferi* infection and ECM. Thus, potential recipients of preventative treatment are easily identified as soon as ECM and/or *B. burgdorferi* infection is determined. Accordingly, as discovered by the present inventors, prophylactic and therapeutic modalities for such complications of Lyme disease are possible.

It has become apparent that, as Lyme disease progresses, successful treatment with antibiotics becomes less reliable because of the persistent presence of the spirochete, coupled with an activated immune system. Therefore, what is needed is a more optimal therapy for the various stages of Lyme disease which can: 1) exploit the mechanism of pathogen binding to the infected host's tissue components; 2) repair and replace host ground substance; or 3) modulate immune activation and/or inflammatory responses.

SUMMARY OF THE INVENTION

The present invention relates to a prophylactic or therapeutic treatment for Lyme disease-associated arthritis (LDAA) in a human or mammalian subject, and includes the step of administering an effective amount of a glycosaminoglycan (GAG) composition to a subject in need thereof, particularly

a polysulfated GAG (PSGAG). In a preferred embodiment, the composition is administered subsequent to the onset of erythema chronicum migrans (ECM).

It is an object of the invention to treat LDAA by administering such compositions to a patient showing joint swelling caused by the presence of *Borrelia burgdorferi* in connective tissues, particularly subsequent to the onset of ECM. The compositions of the present invention may be administered systemically or at the site of swelling, such as by injection at such a site.

In another aspect of the present invention, treatment of LDAA involves coadministering an effective amount of a composition comprising a PSGAG and therapeutic amounts of one or more other compounds that also are effective against *B. burgdorferi*. Particularly contemplated other compounds include antibiotic such as tetracycline, doxycycline, penicillin, amoxicillin, probenecid and ceftriaxone or a combination of antibiotics such as amoxicillin and probenecid. Other contemplated compounds include antibodies, antibody conjugates, chemokine-inhibitors and cytokine inhibitors.

Contemplated PSGAG compounds have a molecular weight range between about 3,000 to 15,000 daltons. A preferred range is about 4,000 to 10,000 daltons and a more preferred range is about 5,000 to 8,000 daltons.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon considering the following detailed description of the invention which describes presently preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. presents a HP-GPC chromatogram for PSGAG.

DETAILED DESCRIPTION

The present invention provides significant improvements in the therapeutic treatment of Lyme disease associated arthritis and associated

inflammatory symptoms, particularly involving connective tissue and joint disorders through the use of product formulations which include semisynthetic polysulfated glycosaminoglycans (PSGAGs). Such compositions have been discovered to possess enhanced biochemical activities for such purposes in comparison to naturally occurring glycosaminoglycans (GAGs).

The present invention provides for a prophylactic treatment for Lyme disease associated arthritis using an injectable form of a PSGAG. PSGAG is a biological polymer with a molecular weight range of about 3,000 to 16,000 Daltons. The invention relates to the use of this select fraction of PSGAG with a distinct molecular weight distribution and poly-ionic properties.

Polysulfated glycosaminoglycan (PSGAG) is a poly-anionic mucopolysaccharide that enhances the inhibition of catabolic enzymes while stimulating anabolic pathways for regeneration of damaged cartilage. Compositions of PSGAG are useful in the prevention and treatment of inflammatory symptoms associated with degenerative joint diseases. Humans and animals infected with Lyme disease caused by the spirochete, *B. burgdorferi*, may suffer from a chronic, destructive arthritis caused by the spirochete as distinguished from classical degenerative joint and connective tissue disorders. The presence of *B. burgdorferi* in the joint triggers an immunological response that results in the release of neutral proteases that degrade the proteoglycans and collagen in cartilage. Due to its unique poly-anionic properties, PSGAG inhibits the attachment of spirochetes, such as *B. burgdorferi*, to joint (connective) tissues. Since PSGAG enhances the inhibition of neutral protease and inhibits the attachment of *B. burgdorferi*, to joint (connective) tissues, PSGAG alone or in combination with a suitable antibiotic may be used to treat Lyme disease associated arthritis.

Treatment comprises systemic administration or direct treatment of specific sites with an effective amount of PSGAG. In a related aspect, treatment comprises co-administration of PSGAG and other compounds that are therapeutically effective against *B. Burgdorferi*. Preferred compounds that are

effective against *B. Burgdorferi* include antibiotics, antibodies and chemokine and cytokine inhibitors.

1. Definitions

"ECM" is a red circular patch that appears usually three days to one month after the bite of an infected tick at the site of the bite. The patch then expands, and sometimes many patches appear that vary in shape. Common sites for such patches are the thigh, groin, trunk and the armpits.

"Co-administered" means that two or more different compositions may be administered to a patient simultaneously or sequentially as long as the compositions are administered in a time frame in which the patient derives therapeutic benefit from such two or more compositions.

"Connective tissue" means any area of the musculoskeletal system where there is a relatively large portion of extracellular matrix in relation to cells. For example, joints are considered connective tissues.

"LDAA" stands for "Lyme Disease Associated Arthritis" and means the intermittent swelling of and pain in a few large joints, especially the knee, which typically recur for several years after infection of patient with the causative microorganism of Lyme disease. In a related aspect, these symptoms occur within weeks to months of onset of erythema chronicum migrans (ECM).

The terms "protective" or "therapeutically effective" generally mean that the PSGAG administered is effective to inhibit significantly the attachment of infective microorganisms to their target tissue or cells in an infected host; or that the administered PSGAG is effective to significantly repair and replace host ground substance in the presence of Lyme disease infection; or to significantly reduce the infected host's immunopathological response to the infection.

"PSGAG" means the semi-synthetic biological polymer composed of repeating disaccharides units of D-glucuronic acid sulfate and N-acetyl-galactosamine 4, 6 polysulfate.

2. Pharmacology of PSGAG:

A. Absorption, distribution, metabolism and excretion of PSGAG after intramuscular administration:

The absorption and distribution of PSGAG after intramuscular injection has been studied in many species including rats, rabbits, humans, horses, and dogs.

In rabbits the maximum blood concentrations occurred 20-40 minutes after intramuscular administration (4, 5). The blood concentration of PSGAG achieved in rabbits was linearly proportional to the dose (1.79-7.50mg/kg) (5).

Following intramuscular administration of PSGAG to human osteoarthritis patients, PSGAG absorption was similar to that in the rabbit (6). The maximum serum concentrations occurred at 30 minutes post injection.

The endogenous distribution of radiolabeled PSGAG after intramuscular (4) and intra-articular (7) injection has been studied in rabbits at 3 and 6 hours and at 1, 2, 3, 7, and 10 days after injection. The results of these investigations were similar and are summarized as follows:

-PSGAG was distributed to all tissues and body fluids investigated

-after intra-articular injection, maximum drug concentrations were found

a) after 3 hours in the adrenals, thyroids, peritoneal fluid, lungs, eyes, aorta, articular cartilage, synovial fluid and spinal cord

b) after 6 hours in kidneys, stomach, testes, adipose tissue, skeletal muscle and brain

c) after 24 hours in liver, spleen, bone marrow, salivary glands, thymus, pancreas, skin and heart.

-the maximum concentration of drug after intra-articular injection of 1 mg/kg in rabbits (7) in the various organs was given at (all values in dpm/mg or mL):

a) kidneys 4782

b) adrenals 439, liver 416, spleen 380, bone marrow 250, thyroids 108, and salivary glands 100

c) thymus 64, pancreas 57, peritoneal fluid 54, lung 51, stomach 47, heart 35, testes 20, adipose tissues 19, eyes 14, and aorta 13

d) articular cartilage and synovial fluid 9 and skeletal muscle 8

e) spinal cord 3 and brain 1.

Several studies in humans have established details of the distribution of PSGAG to blood and joint tissues following intramuscular injection.

PSGAG is bound to serum proteins in human blood (6,8,9). The drug binds to both albumin and chi- and beta-globulins and the extent of the binding is stated to be 30-40% (8). Thus, the drug exists in both the bound and free form in the blood stream (8).

After intramuscular injection in man, peak blood levels occur in about 30 minutes then decrease rapidly for about 24 hours to a level that remains relatively constant for several days. This can be explained by the distribution of the unbound drug to tissues (6,8) and the persistence of the bound drug in the blood.

Because of its relatively low molecular weight (average 10,000 Daltons: range about 2,000-16,000 Daltons), the synovial membrane is not a significant barrier to distribution of PSGAG from the blood stream to the synovial fluid (4,6,8,9,10). Distribution from the synovial fluid to the cartilage takes place by diffusion (11). In the articular cartilage, the drug is deposited into the cartilage matrix (11) and appears to be bound to macromolecules; perhaps to proteoglycans (11) or other non-collagenous proteins (1).

In a study of the distribution of PSGAG to articular cartilage of man after a single intramuscular injection (125 mg ^{3}H-PSGAG), maximum drug concentrations reached all layers of the articular cartilage 24 to 48 hours after injection and decreased steadily from 48 to 96 hours (6). The uptake of the drug

in cartilage appears to vary in the various layers of cartilage with the highest uptake by the more superficial layer and the lowest uptake in the layer nearest subchondral bone. A summary of the data from this study is presented in Table 1.

TABLE 1

PSGAG Concentrations in Cartilage after Intramuscular Injection of 125 mg to Human Patients (ug/g)

Time Deep Layer	N	Surface Layer	Middle Layer	Deep Layer
12 h	6	2.52 ± 1.53	2.18 ± 1.43	1.71 ± 1.04
24 h	7	4.43 ± 2.55	2.99 ± 1.09	1.98 ± 0.79
48 h	6	4.90 ± 4.13	2.13 ± 0.96	1.61 ± 0.57
72 h	4	2.33 ± 0.79	2.08 ± 0.77	1.83 ± 0.74
96 h	2	1.71 ± 0.12	1.04 ± 0.10	0.99 ± 0.15

In an equine study the mean cartilage concentration of PSGAG in 4 horses 96 hours after receiving 500 mg ^{3}H-PSGAG by intramuscular injection was approximately 0.3 ug/g and the distribution curves in serum and synovial fluid were similar to those found in human and rabbit studies (12) at similar doses.

In a canine study the mean cartilage concentration of PSGAG in 9 dogs 72 hours after intramuscular injection of 2 mg ^{3}H-PSGAG/lb body weight was approximately 0.15 ug/g. Again the serum and synovial fluid distribution curves were consistent with those found in humans and other species (data on file).

The uptake of PSGAG in joint tissues appears to be dependent on the condition of the tissues with the drug showing a predilection for inflamed or diseased tissue. It has been demonstrated using an adjuvant model of inflammation in the paw of the rat that the drug reaches higher concentrations in inflamed joint tissue (8). In equine studies, peak synovial fluid levels of

PSGAG were approximately 30% higher in joints with surgically induced full thickness cartilage defects (12) than in non-traumatized control joints. In dog cartilage from joints with adjuvant induced synovitis, there was an 80% higher level of PSGAG than in cartilage from normal joints 72 hours after intramuscular injection of 2 mg ^{3}H-PSGAG/lb body weight.

Metabolism of PSGAG in humans and rabbits includes desulfation and depolymerization (4, 6, 7, 9); metabolites include sulfate, oligosaccharide and monosaccharides. In rabbits, metabolism is reported to take place in the liver, spleen, and bone marrow (7). Metabolism may also occur in the kidneys (13). No metabolism of the drug has been observed in the knee joint of rats (16). It is, however, known that glycosaminoglycans are metabolized by chondrocytes. It has been postulated that PSGAG can be degraded in granulocytes (13) and that extracellular degradation may take place (14).

In rabbits, metabolism of PSGAG begins within 3 hours of intra-articular injection and metabolites may be excreted for several days (7). This pattern also occurs after intramuscular injection (4). The rate of degradation of PSGAG in humans is reported to be similar to that in rabbits (6, 8).

PSGAG administered intramuscularly and not protein bound or bound to other tissues is excreted (7). This excretion is primarily via the kidneys, with a very small proportion in the feces. In rabbits, 55.3% of intramuscularly administered PSGAG was excreted in the urine and 1.7 % in the feces within 2 days (4). In humans, 35-40% of a 50 mg intramuscular dose of PSGAG was excreted via the kidneys within 12 hours of administration (6).

Both metabolites and the unmetabolized drug are excreted and the proportion of metabolites increases with time (4, 7, 8, 9).

B. Biochemical Effects of PSGAG in Degenerative Joint Diseases

At therapeutic doses, PSGAG has several biochemical effects on injured or degenerative joints. The sum of these effects is called chondroprotection: the inhibition of degradation and effects favorable to repair

of articular cartilage (1). Drugs having these effects are classified as chondroprotective.

1. Incorporation of PSGAG in cartilage matrix

This effect has been described in a number of reports (10,11,15,16). PSGAG binds to macromolecules in the extracellular matrix of cartilage; the identity of the binding site is thought to be either a proteoglycan (16) or other noncollagenous protein (1). This activity in damaged cartilage with a depleted proteoglycan content has been described as a repair process (17,18,19). It is possible that this binding effect may improve the biochemical properties of damaged cartilage matrix, such as water binding capacity, or may protect damaged matrix components from further enzymatic degradation.

2. Inhibition of Catabolic Enzymes

The ability of PSGAG to inhibit a broad range of catabolic enzymes has been reported in both *in vitro* and *in vivo* test systems. This is significant because enzymatic degradation of collagen, proteoglycans, and hyaluronic acid is a critical element of the pathogenesis of degenerative joint disease (1). Many of the most important of these catabolic enzymes are inhibited by fluid or tissue concentrations of PSGAG as low as about 0.1 to 2 ug/mL in some cases (1,4).

The mechanism of enzyme inhibiting varies with the type of enzyme. For many of the glycosidases, which degrade glycosaminoglycans and hyaluronic acid, the inhibition is competitive; the drug acts as a competitive substrate for the enzyme (13). For elastase and other lysosomal peptidases the inhibition appears to be both competitive and noncompetitive. In the case of elastase, electrostatic binding of the highly positive enzyme molecule to the negatively charged PSGAG molecule inactivates the enzyme (20). In the case of certain neutral proteases, PSGAG appears to inhibit the conversion of the enzymes from inactive to active form. (21).

The inhibition of more than a dozen catabolic enzymes by PSGAG has been reported. Five of the most important of these are specifically described below.

The inhibition of elastase has been reported *in vitro* (14,20,22,23). Inhibition was described at concentrations as low as 0.5 ug/mL. Elastase has been shown to degrade elastin, collagen, proteoglycans, and structural glycoproteins (20).

Stromelysin is a neutral protease which degrades proteoglycans at low concentrations. In nutritionally deprived equine synoviocyte cultures, PSGAG was the only drug tested which significantly inhibited stromelysin at concentrations readily achievable in equine joint tissues (24). In an in vitro study of purified rat stromelysin, PSGAG at concentrations of 0.05-0.5 ug/mL inhibited the activity of the enzyme (41).

In an *in vivo* study in dogs with transected anterior cruciate ligaments, the levels of active metalloprotease in articular cartilage were significantly lower in dogs which had received 4 mg/kg PSGAG by intramuscular injection twice weekly for 4 weeks when compared to the cartilage of control dogs (21). The metalloproteases have a potent capacity to degrade proteoglycans.

The ability of PSGAG to inhibit collagen degrading enzymes has been demonstrated. These enzymes include cathepsin Bi (14,25,26) and elastase (see above).

PSGAG also inhibits enzymes which degrade hyaluronic acid; the glycoanohydrolases or "hyaluronidases" (13,27,28,29), betaglucuronidase (13,27,28,29,30), and beta-N-acetylglucosaminodases (13,18,28,30). Loss of hyaluronic acid in synovial fluid and in the proteoglycan complexes of cartilage matrix due to enzymatic activity are significant mechanisms in the pathogenesis of degenerative joint diseases (1).

It has thus been demonstrated that PSGAG inhibits enzymes which destroy hyaluronic acid, proteoglycans and collagen. Degenerative joint disease is characterized by a net loss of these critical components (1).

3. Anabolic Effects in Damaged or Diseased Joint Tissue

PSGAG has been reported to stimulate the endogenous synthesis of collagen, proteoglycans and hyaluronic acid by chondrocytes or synoviocytes. The stimulation of proteoglycan and hyaluronate in increased amounts and with higher molecular weight by PSGAG in cultured human chondrocytes has been reported (32). These studies were confirmed in lapine chondrocytes by another investigator (1).

Elevation of both proteoglycan and collagen synthesis have been demonstrated in embryonic chicken cartilage (33). These results have been confirmed in other investigations (33,34) in cultures of osteoarthritic human cartilage and in cultures of arthritic equine cartilage (35).

Strong stimulation of hyaluronic acid synthesis by synoviocytes has been demonstrated both *in vitro* and *in vivo*. In a synovial membrane explant culture, PSGAG stimulated hyaluronic acid synthesis at concentrations as low as 0.2 ug/mL (36). Other investigations in synovioblast cultures have confirmed these findings (31,32,37). An increase in synovial fluid hyaluronate concentration has been demonstrated *in vivo* in rabbit (36), humans (31), pigs (38), and horses (12) receiving PSGAG therapy.

The combination of the incorporation of PSGAG into cartilage matrix, the inhibition of catabolic enzymes and the stimulation of endogenous anabolic pathways in joint tissue result in an attenuation of the overall net loss of collagen and proteoglycans characteristic of degenerative joint disease. These actions are the hallmark of a chondroprotective drug.

4. Anti-Inflammatory Effects

In addition to the effects previously described, PSGAG has also been shown to be anti-inflammatory in at least two important pathways. In an *in vitro* cell culture system, PSGAG was shown to inhibit the biosynthesis of the eicosanoid prostaglandin E2 (39). This potent inflammatory mediator plays an important role in pain and inflammation in joint disease (1). A dose dependent

decrease in the release of toxic oxygen radicals from human neutrophils by PSGAG has been reported (40). Toxic oxygen radicals are implicated in the early breakdown of hyaluronic acid in the synovial fluid of inflamed joints (1). These anti-inflammatory effects may account in part for the relief of pain and inflammation seen as a result of treatment of degenerative joint diseases with PSGAG.

5. <u>Inhibition of *B. burgdorferi* binding to joint (connective) tissues</u>

Humans and animals infected with Lyme disease caused by the spirochete, *B. burgdorferi*, may suffer from a chronic, destructive arthritis. *B. burgdorferi*, has been found to infect multiple tissues where the spirochete binds with the host cell proteoglycans. Proteoglycans consist of core proteins linked with negatively charged glycosaminoglycans. Due to its binding to host cells, antibiotic therapy against the Lyme disease spirochete is ineffective and a persistent infection often begins after the first year of infection.

An *in vitro* study of the proteoglycan binding of *B. burgdorferi* found that dextran sulfate and heparin sulfate inhibited the attachment of *B. burgdorferi* to mammalian cells (41). The present inventors have discovered that PSGAG also will inhibit attachment of spirochetes, such as *B. burgdorferi*, to joint (connective) tissues.

3. Treatment of Lyme Disease

Lyme disease may be treated by various therapeutic methods that include the administration of PSGAG. It is contemplated that PSGAG may be coadministered or combined in dosage forms with other anti-Lyme agents including antibiotics, anti-inflammatories, antibodies against *B. Burgdorferi* epitopes and antibodies against cytokines and chemokines. In a preferred embodiment, PSGAG may be co-administered with the following antibiotics: tetracycline, deoxycycline, penicillin, amoxicillin, ceftriaxone and probenecid.

For example, antibodies against *B. burgdorferi* may be useful in the treatment of this disease, particularly in combination with the administration of PSGAG. Useful antibodies may be generated by standard methods. For example, polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies. These antibodies can be, for example, polyclonal or monoclonal antibodies, as well as chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments. In a preferred embodiment, antibodies may be generated which comprise antibody conjugates (U.S. Patent No. 5,612,016).

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, *Nature*, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). All of these techniques are incorporated herein by reference. Techniques described in U.S. Patent No. 4,946,778, incorporated herein by reference, for the production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention.

In a preferred embodiment of the present invention, antibodies to cytokines or cytokines can be co-administered with PSGAG. Cytokines or chemokines can be obtained from any source, most preferably from humans. In preferred embodiments, the following chemokines are envisaged: RANTES (U.S. Patent No. 5,451, 660), IP-10 and mig (U.S. Patent No. 5,871, 723), MCP-1 (U.S. Patent No. 5,707,815), gro-alpha and IL-8 (U.S. Patent No. 5,831,032).

In a related aspect, the cytokine IL-12 is preferred. For example, for treatment of humans, antibodies to IL-12, in soluble form, would typically be administered in a single dosage of between 10 mg and 20 mg/kg of body weight. For oral administration, 500 mg to 1000 mg can be given P.O. For parenteral administration, 10 mg to 20 mg/kg of body weight can be administered as a single or as a weekly intravenous injection. However, the age, weight and condition of the individual must be considered in determining a final dose. For administration of antibodies to IL-12 in particulate form, 500 mg to 1000 mg can be microencapsulated as described for slow release over a four to eight week period. One skilled in the art will realize that dosages are best optimized by the practicing physician and methods for determining dosages are described for example, in Remington's Pharmaceutical Sciences (24).

Suitable carriers for oral administration of antibodies to cytokines or lymphokines include one or more substances which may also act as flavoring agents, lubricants, suspending agents, or as protectants. Suitable solid carriers include calcium phosphate, calcium carbonate, magnesium stearate, sugars, starch, gelatin, cellulose, carboxypolymethylene, or cyclodextrans. Suitable liquid carriers may be water, pharmaceutically accepted oils, or a mixture of both. The liquid can also contain other suitable pharmaceutical additions such as buffers, preservatives, flavoring agents, viscosity or osmo-regulators, stabilizers or suspending agents. Examples of suitable liquid carriers include water with or without various additives, including carboxypolymethylene as a pH-regulated gel. The antibodies may be contained in enteric coated capsules that release antibodies into the intestine to avoid gastric breakdown.

In a preferred embodiment antibodies are directed to OspA, OspB (U.S. Patent No. 5,747,294), OspE, OspF and S1 (U.S. Patent No. 5,807,685) are co-administered with PSGAG. For example, Anti-OspA and anti-OspB polypeptide antibodies of this invention may be generated by infection of a mammalian host with B. burgdorferi, or by immunization of a mammalian host with an OspA or OspB polypeptide. Such antibodies may be polyclonal or

monoclonal, it is preferred that they are monoclonal. Methods to produce polyclonal and monoclonal antibodies are well known to those of skill in the art. For a review of such methods, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, ed. E. Harlow and D. Lane (1988), and D. E. Yelton, *et al.*, *Ann. Rev. of Biochem.*, 50, pp. 657-80 (1981).

In a further preferred embodiment, OspA and OspB antibodies are combined as pharmaceutical compositions with carriers and adjuvants in their dosage forms. The pharmaceutical compositions of OspA and OspB antibodies may be in a variety of conventional depot forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, capsules, suppositories, injectable and infusible solutions. The preferred form depends upon the intended mode of administration and prophylactic application.

Such dosage forms may include pharmaceutically acceptable carriers and adjuvants which are known to those of skill in the art. These carriers and adjuvants include, for example, RIBI, ISCOM, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances, such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and polyethylene glycol. Adjuvants for topical or gel base forms may be selected from the group consisting of sodium carboxymethylcellulose, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wood wax alcohols.

In light of the foregoing general discussion, the specific examples presented below are illustrative only and are not intended to limit the scope of the invention. Other generic and specific configurations will be apparent to one skilled in the art.

EXAMPLES

Example 1. Polysulfated Glycosaminoglycan Production

A. Intermediate #1A "Purified Cartilage"

1. Mincing: Frozen cartilage is minced. The source of the cartilage is bovine trachea.

2. Degreasing: The minced cartilage is heated in hot water (80°C) for twenty minutes. The cartilage's fat is separated from the hot tissue via centrifugation. 2400 kg of frozen cartilage is reduced to 1800 kg after this process. It has lost 25% of its weight.

3. Drying : The moist granules of degreased cartilage are dehydrated using hot air (80°C) for 22 hours. The 1700 kg is further reduced to 500 kg. This represents a 60% loss in weight.

4. In-Process Specifications : After completion of the drying process, Intermediate 1 A is sampled and tested by Quality Control for:

TEST	LIMITS
Loss on Drying	Not more than 8%
Fat Content	Not more than 7%

If within limits the material is released for further processing.

B. Intermediate #1 "Treated Cartilage"

1. Formaldehyde Treatment :500 kg of intermediate 1 A - 54.65 kg (50.6 liters) is weighted out, added and the mixture then agitated for twenty minutes. The treated cartilage is transferred to PVC drums and stored for 4-9 weeks at room temperature.

2. *In-Process Specifications* :The only in-process test is for microbial limits. The limit should be less than 10 micro-organisms per gram with the absence of any harmful species.

C. Intermediate #2 "Extraction of Glycosaminoglycan"

1. Alkaline Extraction :A dilute alkaline solution of sodium hydroxide is heated to 37 degrees centigrade, 270 kg of intermediate 1 is added and the suspension is agitated for 22-27 hours at 36-38°C.

SEPARATION OF SOLIDS: Fatty material is skimmed from the surface of the suspension. The turbid solution is transferred to a reservoir with the moist solids of the tissue remaining (which are discarded). The turbid solution passes through a in-line separator which removes the suspended particles.

NEUTRALIZATION: The extract is transferred to another vessel and the pH is adjusted to 7.2 with hydrochloric acid. The extract is then clarified by filtration.

EVAPORATION: A volume of about 5600 liters is evaporated under a vacuum to a volume of 400-480 liters.

SOLVENT PRECIPITATION: Sodium chloride is added to the reduced extract. The pH is adjusted to 7.5 with hydrochloric acid. Four volumes of methanol is added to one volume of extract. The product is allowed to precipitate and settle overnight. The next day the supernate liquid is drawn from the top of the vessel and the precipitated product is suspended in the remaining liquid.

CENTRIFUGATION: The suspension is centrifuged at 400 rpm. The precipitate is collected on polypropylene filter cloth.

DRYING: The precipitate is dried for three hours at 80°C at atmospheric pressure, then under vacuum for 64 hours.

GRINDING: 25 kg portions are ground in ball mills for 38 hours. The balls are removed and the powder transferred to drums lined with double polyethylene bags.

IN-PROCESS SPECIFICATIONS: Intermediate 2 is Q.C. tested for the following:

TEST	LIMITS
Loss on Drying	Not more than 4%
Nitrogen	Not more than 3.2%
Hexuronic Acid	Between 27.0 and 36.9%w/w
Protein	Less than 0.1%

If within limits the material is released for further processing.

D. Intermediate #3 "Sulfuric Esterification Process"

SULFURIC ESTERIFICATION: A pyridine suspension of intermediate 2 is added to a reaction vessel. Chlorosulfonic acid is slowly added to the vessel via a dosing device. The reaction causes the temperature to rise to 98°C. The mixture is agitated for one hour with the temperature maintained at 75-90°C.

ACID SOLVENT PRECIPITATION: The hot reaction mixture is added to 260 liters of methanol and mixed for 5 minutes. A precipitate settles and the supernatant is withdrawn. Sodium chloride is added and the precipitate is dissolved in hot water to a volume of 160 liters. 320 liters of methanol is added and the PSGAG precipitates again. The supernatant is again withdrawn.

ALKALINE SOLVENT PRECIPITATION/CENTRIFUGATION: Sodium chloride and water is added to dissolve the precipitate. The pH is adjusted to 11.5 with sodium hydroxide and the Polysulfated Glycosaminoglycan precipitated via the addition of methanol. After settling

overnight the supernatant is withdrawn and the precipitate is suspended in the remaining solution. The suspension is centrifuged under a continuous stream of nitrogen. The precipitate is removed and the process repeated three times.

FIRST DRYING: The third precipitated is washed with 250 liters of methanol and the filter cake is dried in a fluid bed drier using hot air at 85°C.

FILTRATION: The dried product is dissolved in a sufficient amount of water. The pH is adjusted to 7.0 and after standing overnight the solution is filtered.

PRECIPITATION FROM NEUTRAL SOLUTION: Four volumes of methanol is added to one volume of filtrate. The pH is maintained at 7. The precipitate is allowed to settle for at least 3 hours. The supernatant is removed and the suspension centrifuged under a continuous stream of nitrogen. The filter cake is rinsed with methanol.

SECOND DRYING: The filter is dried in the fluid bed drier using hot air. The dried precipitate is collected in PVC drums lined with double polyethylene bags.

IN-PROCESS SPECIFICATIONS: Intermediate 3 is Q.C. tested for the following:

TEST	LIMITS
Identification	Must comply
Loss on drying	Not more than 10%
Nitrogen	Between 1.4 and 2.1% w/w
Sulfate Content	Between 36.5 and 46.5% w/w
Hexuronic Acid	More than 17.6% w/w

If within limits the material is released for further processing.

E. Intermediate #4A "Depolymerization"

1. Depolymerization: Sufficient amounts of Intermediate 3 and sodium chloride are dissolved in water. EDTA and sodium hydroxide are added. The pH is adjusted to 6.05 and the solution is heated to 90°C. To the hot solution, a hydrogen peroxide solution is stirred in and the temperature maintained for 115 minutes. The solution is then cooled to 25°C.

2. Precipitation/Drying: The solution is transferred to another vessel. Sodium chloride is added and the Polysulfated Glycosaminoglycan is again precipitated using methanol. The methanol is recovered and the precipitate collected via filtration. The precipitate is dried as described (IV) 4 above.

3. *In-Process Specifications*: Intermediate 4 A is Q.C. tested for the following:

TEST	LIMITS
Identification	Must comply
Loss on Drying	Less than 10%

If within limits the material is released for further processing.

F. Intermediate #4 "Molecular Size Tailoring"

1. Fractionation: Intermediate 4 A is dissolved in hot water (60°C). An appropriate amount of peracetic acid is added and the solution stands for 180 minutes, after which it is cooled to 25°C over a thirty minute period.

Sodium chloride is added and the pH is adjusted to 10.5 with sodium hydroxide. The solution is brought to volume and the viscosity measured. Based on the viscosity an appropriate amount of methanol is calculated. This first addition of methanol precipitates out unwanted fractions of Polysulfated Glycosaminoglycan. Therefore after the addition of the methanol, the solution

sits overnight and then the supernatant is withdrawn and transferred to another vessel where additional methanol is added to precipitate out the desired fraction of Polysulfated Glycosaminoglycan. The supernatant methanol is recovered and the precipitated Polysulfated Glycosaminoglycan collected via filtration.

2. Drying: The precipitate is dried at 50°C for twelve hours under a constant vacuum. Two lots are then mixed and dried for one hour in a double cone drier. The mixed lot is the stored in drums lined with double polyethylene bags.

3. In-process specifications: Intermediate 4 is Q.C. tested for the following:

TEST	LIMITS
Identification	Must comply
Mean Molecular Weight	Between 4,000 -7,000 Daltons

If within limits the material is released for further processing.

G. Intermediate #5 "Desalting"

1. First filtration: Intermediate 4 is dissolved in water and filtered.

2. Ion exchange treatment: The solution is filtered through a glass column containing a cation exchange resin. The void volume is discarded and a total of 440 liters of filtrate collected.

3. Acid precipitation: The solution is split and transferred to two separate vessels. Sodium chloride is added to each vessel and the Polysulfated Glycosaminoglycan is again precipitated using methanol. The methanol is recovered and the precipitate collected via filtration. The steps "Filtration", "Ion Exchange Treatment" and "Acid Precipitation" are repeated two more times.

4. Neutral precipitation: Sodium chloride is added and the pH is adjusted to 7.5 with sodium hydroxide. A sufficient amount of methanol is

added to precipitate the Polysulfated Glycosaminoglycan. The precipitate is recovered via filtration.

5. *Second filtration*: The filter cake is dissolved in situ within the closed filter with distilled water. The resulting solution is filtered through a 0.45 micron filter which is integrity tested both before and after use via the bubble point method.

6. *Drying*: The solution is fed into a spray drier using HEPA filtered hot air (85°C). The dried Polysulfated Glycosaminoglycan is stored in drums lined with double polyethylene bags.

7. *In-process specifications*: Intermediate 5 is Q.C. tested for the following:

TEST	LIMITS
Identification	Must comply
Loss on Drying	Less than 10% w/w
Absorption at 400 nm	Less than 0.05 abs. units

If within limits the material is released for further processing.

H. Final Product "Purification"

1. *Treatment with activated charcoal*: Intermediate 5 is dissolved in distilled water. Activated charcoal is added, the suspension is agitated and heated to 90°C for one hour.

2. *Filtration*: The hot solution with the suspended charcoal is pumped through a filter to a second vessel and combined with another sublot processed in a similar manner.

3. *First membrane filtration*: Sodium chloride and EDTA are added to the solution. The pH is adjusted to 7.5 with hydrochloric acid. The solution is filtered through an in-line combination of a plate filter and a 0.2 micron

cartridge membrane filter. The membrane filter should be integrity tested prior to and after use.

4. First solvent precipitation: Two membrane filtrates manufactured as described above are combined and precipitated with three volumes of methanol in a stainless steel vessel under constant agitation. After one hour the Polysulfated Glycosaminoglycan is recovered via filtration and washed with methanol. The filtrate is then redissolved in distilled water and more sodium chloride added.

5. Final solvent precipitation: The solution from the last step is diluted to 400 liters with distilled water. 2660 liters of methanol is added with stirring. After one hour of agitation the total suspension is filtered with a closed pressure filter. The filter cake is washed five times with 20 liter portions of methanol. The filtrate is then redissolved in distilled water and the volume brought to 200 liters.

6. Second membrane filtration: The solution is filtered with a steam-sterilized cartridge membrane filter equipment into a sterile buffer vessel, then combined with 50 liter distilled water washings of the filter housing. The pH is adjusted to 7.5.

7. Drying: The solution is fed to a spray drier, with an air entrance temperature at 140 and an exit temperature of 85°C. The dried product is collected in a drum lined with double polyethylene bags, then transferred to the quarantine area for sampling and examination by Q.C. The yield of the final product is about 64 to 72 kg of white powder.

8. FINAL PRODUCT SPECIFICATIONS :The final product is Q.C. tested for the following:

TEST	LIMITS
Description	Must comply
Identification	Must comply

TEST	LIMITS
Molecular Weight Determination	Mw: Between 6,000 and 10,000 daltons Mn: Between 4,000 and 8,000 daltons Mw/Mn: Not more than 1.5
Loss on Drying	Less than 10% w/w
Protein	Not more than 0.05% w/w
Optical Rotation	Between -7° and -17°
pH	Between 5.0 and 8.0
Heavy Metals	Not more than 0.003%
Hexuronic Acid	Between 18.1 and 24.5%
Hexosamine	Between 21.0 and 28.0%
Sulfate	Between 36.7 and 44.7%
Chloride	Not more than 750 ppm
Pyridine	Not more than 20 ppm
Methanol	Not more than 0.25%
Electrophoresis	On the strip only one violet-blue spot is seen at a distance of about 3 cm from the starting point
Microbial Limits	Not more than 50 micro-organisms per gram. Absence of Salmonella, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa
Yeast and molds	Not more than 50 yeast or molds per gram
Bacterial Endotoxins	Not more than 0.5 EU per mg

If within limits the material is released and suitable for injectable use.

Example 2. <u>Characterization of the PSGAG Compounds</u>

The unique poly-anionic properties of PSGAG is determined by electrophoresis. A 70 mm cellulose acetate (CA) membrane, saturated with 0.2 N hydrochloric acid is suspended with contact between the cathode and anode

of an electrophoresis chamber. A test solution of PSGAG containing about 1 microgram of PSGAG is applied to the cathode end of the strip. A current of about 65 mA is applied for 65 minutes. The CA membrane is removed from the tank, then stained with a 0.1% toluidine blue solution for 5 minutes. The CA membrane is rinsed with a sufficient quantity of water to remove the excess toluidine blue. On the strip, PSGAG appears as only one violet-blue spot at a distance of 3 cm from its starting point.

The cathode is the negative terminal of the electrophoresis chamber. Anionic compounds, such as PSGAG, under an applied current will migrate towards the chamber's anode (i.e., its positive terminal). The poly-anionic property of PSGAG was compared to hyaluronic acid, chondroitin sulfate A, chondroitin sulfate B (dermatan sulfate), chondroitin sulfate C, heparin sulfate and keratan sulfate using electrophoresis. The results are as follows:

SUBSTANCE	DISTANCE (CM)
PSGAG	3.1 ± 0.3 (n=12)
Hyaluronic Acid	1.1 ± 0.1 (n=2)
Chondroitin Sulfate A	2.1 ± 0.0 (n=2)
Dermatan Sulfate	1.7 ± 0.0 (n=2)
Chondroitin Sulfate C	2.0 ± 0.3 (n=2)
Heparin Sulfate	2.3 ± 0.0 (n=2)
Keratin Sulfate	2.5 ± 0.1 (n=2)

The results demonstrate that the anionic property of PSGAG is significantly greater than that of hyaluronic acid, chondroitin sulfate A, dermatan sulfate, chondroitin sulfate C, heparin sulfate and keratan sulfate. PSGAG's strong negative charge enables it to bind to positively charged moieties of proteins.

The molecular weight distribution of PSGAG is determined by High Pressure Gel Permeation Chromatography (HP-GPC). The HP-GPC system uses a high efficiency gel filtration column packed with packed with a bonded diol-coated silica gel with a particle size of 7 microns. The column's molecular weight range is between 100 to 50,000 Daltons. Through this column, a mobile phase consisting of a dilute solution of sodium sulfate (0.05 M) is pumped at a constant flow rate, pressure and temperature. Samples of the PSGAG are diluted to 5 mg per mL with the mobile phase and a portion of the resulting solution (10 µL) is injected into the HP-GPC system.

As each separated substance is eluted, a refractive index detector measures the substance's peak response which is proportional to the substance's concentration. A computerized GPC software program interprets the data and calculates the relative: weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity index (M_w/M_n) of the sample. These values are based on commercially available molecular weight standards ranging from 1,000 to 47,300 Daltons which are used to calibrate the system immediately prior to sample analysis.

A typical HP-GPC chromatogram for PSGAG is contained in ***Figure 1***. PSGAG is a semi-synthetic biological polymer composed of repeating disaccharide units of D-glucuronic acid sulfate and N-acetyl-D-galactosamine 4,6 polysulfate. It exhibits a uniform molecular weight distribution curve characterized as follows:

Mw:	Between 6,000 and 10,000 Daltons
Mn:	Between 4,000 and 8,000 Daltons
Mw/Mn:	Note more than 1.5

Example 3. Formulation of PSGAG Composition into a Pharmaceutical Product

The preferred dosage form for human use is as a sterile, isotonic solution prepared by dissolving a suitable quantity of PSGAG in water for injection, where the final concentration of PSGAG is between about 50 mg/mL and about 125 mg/mL of PSGAG, preferably between about 75 mg/mL and about 100 mg/mL. A sufficient amount of sodium chloride is added to adjust the solution's osmolality to an isotonic range of about 270 to about 330 mOmol/kg. To prepare a multiple dose injection, suitable antimicrobial preservatives, such as benzyl alcohol, methyl paraben and/or propyl paraben may be added at effective concentrations during formulation. The bulk solution is sterilized via filtration though a 0.2 micron membrane filter, then aseptically filled into suitable sterile, pyrogen free ampules or vials.

The content of PSGAG in the injection is determined by its hexuronic acid content. PSGAG is composed of repeating disaccharide units of D-glucuronic acid sulfate and N-acetyl-D-galactosamine 4,6 polysulfate. Upon acid digestion, the D-glucuronic acid sulfate contained in PSGAG's repeating disaccharide units is hydrolyzed to hexuronic acid. Based on its hexuronic acid content, the injection contains about 90.0 to about 110.0% of its labeled amount of PSGAG.

In order to provide an injectable solution suitable for human use, the protein content and endotoxin content in PSGAG has been reduced from 0.1% w/w and 2.5 EU /mg respectively to 0.05% and 0.5 EU/mg.

Example 4. Treatment of Human Lyme Disease Patients

The preferred method of treating inflammatory symptoms associated with the chronic, destructive arthritis caused by the spirochete, *B. burgdorferi*, is a suitable injectable formulation of PSGAG administered intra-articularly, intramuscularly, subcutaneously, or by local infiltration at a dose of about 0.5 mg to about 5 mg , preferably about 1 mg to about 4 mg, or more preferably

about 2 mg to about 3 mg PSGAG per kilogram body weight twice a week over a seven to eight week period. Where the PSGAG dosage in mg per kilogram body weight administered is sufficient to provide a minimum therapeutic level of about 0.1 μg/mL of PSGAG throughout its seven to eight week treatment regime. Since upon administration, PSGAG is distributed to all tissues and body fluids, this minimum therapeutic level of about 0.1 μg/mL throughout its seven to eight week treatment regime will aid in the inhibition of the attachment of *B. burgdorferi*, to joint and other connective tissues. Therefore, PSGAG alone or in combination with a suitable antibiotic may be used to inhibit the attachment of *B. burgdorferi*, to joint and other connective tissues.

It should be understood that the foregoing discussion and examples merely present a detailed description of certain preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. All articles, patents and patent applications that are identified in this patent application are incorporated by reference in their entirety.

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CLAIMS

1. A prophylactic or therapeutic treatment for Lyme disease-associated arthritis (LDAA) in a subject, comprising the step of administering an effective amount of a glycosaminoglycan (GAG) composition to a patient in need thereof.

2. The method of claim 1, wherein the patient is administered the GAG composition subsequent to the onset of erythema chronicum migrans (ECM).

3. The method of claim 1, wherein the GAG of the composition is polysulfated-glycosaminoglycan (PSGAG).

4. The method of claim 1, wherein said treatment comprises administering an effective amount of said GAG composition to a patient showing joint swelling caused by the presence of *Borrelia burgdorferi* in connective tissues.

5. The method of claim 4, wherein said GAG composition further comprises an antibiotic and an antibody against *B. burgdorferi*.

6. The method of claim 4, wherein the patient is administered the GAG composition subsequent to the onset of ECM.

7. The method of claim 4, wherein the GAG of the composition is PSGAG.

8. The method of claim 4, wherein the GAG composition is administered systemically or at the site of swelling.

9. The method of claim 8, wherein the GAG composition is administered at the site of swelling by injection.

10. A method of treating LDAA in a patient showing joint swelling due to infection by *B. burgdorferi* in connective tissue, comprising the step of coadministering an effective amount of a composition comprising a GAG and a compound which is effective against *B. burgdorferi*.

11. The method of claim 10, wherein the patient is administered the composition subsequent to the onset of ECM.

12. The method of claim 10, wherein the GAG of the composition is PSGAG.

13. The method of claim 10, wherein the compound effective against *B. burgdorferi* is an antibiotic.

14. The method of claim 13, wherein the antibiotic is selected from the group consisting of tetracycline, doxycycline, penicillin, amoxicillin, probenecid and ceftriaxone.

15. The method of claim 14, wherein the antibiotic is penicillin, tetracycline or doxycycline.

16. The method of claim 13, wherein the antibiotic is a combination of amoxicillin and probenecid.

17. The method of claim 14, wherein the antibiotic is ceftriaxone.

18. The method of claim 10, wherein the compound effective against *B. burgdorferi* is selected from the group consisting of an antibody, an antibody conjugate, a chemokine-inhibitor and a cytokine inhibitor.

19. The method of claim 18, wherein the compound effective against *B. burgdorferi* is an antibody.

20. The method of claim 18, wherein the compound effective against *B. burgdorferi* is an antibody conjugate.

21. The method of claim 19, wherein the epitope is selected from the group consisting of OSPA, OSPB, OSPE, OSPF and S1.

22. The method of claim 20, wherein the epitope is selected from the group consisting of OSPA, OSPB, OSPE, OSPF and S1.

23. A composition comprising GAG, wherein said GAG is a PSGAG, with a molecular weight range between 4,000 and 10,000 Daltons.

24. The GAG composition of claim 23, further comprising an antibiotic.

25. The composition of claim 24, wherein the antibiotic is selected from the group consisting of tetracycline, doxycycline, penicillin, amoxicillin, probenecid and ceftriaxone.

26. The composition of claim 25, wherein the antibiotic is penicillin, tetracycline or doxycillin.

27. The composition of claim 24, wherein the antibiotic is a combination of amoxicillin and probenecid.

28. The composition of claim 25, wherein the antibiotic is ceftriaxone.

29. The composition of claim 24, further comprising an antibody against *B. burgdorferi*.

30. The composition of claim 24, further comprising an antibody conjugate against *B. burgdorferi*.

31. The composition of claim 24, further comprising a chemokine inhibitor.

32. The composition of claim 24, further comprising a cytokine inhibitor.

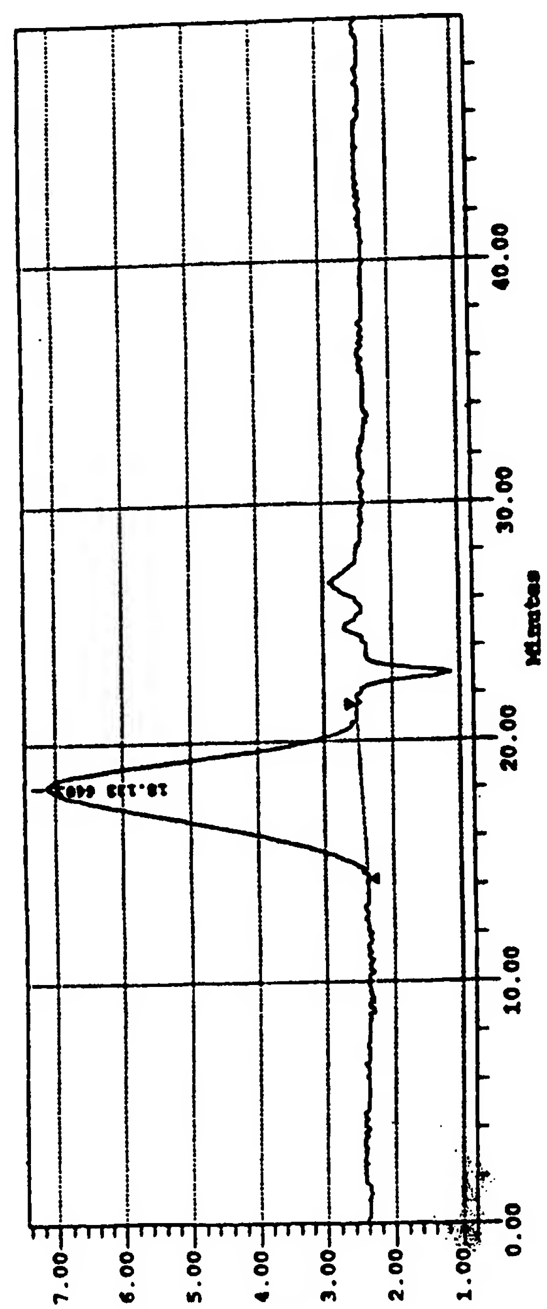

FIG. 1